

IDENTIFICATION OF TELOMERASE IN DOG

A Senior Honors Thesis

by

ANNA LYUKSYUTOVA

**Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
in partial fulfillment of the requirements of the**

**UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS**

April 2000

Group: Cell Biology 1

IDENTIFICATION OF TELOMERASE IN DOG

A Senior Honors Thesis

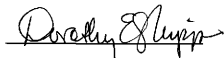
By

ANNA LYUKSYUTOVA

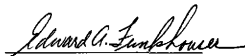
Submitted to the Office of Honors Programs
& Academic Scholarships
Texas &M University
In partial fulfillment of the Designation of

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOW

Approved as to style and content by:



Dorothy Shippen
(Fellows Advisor)



Edward A. Funkhouser
(Executive Director)

April 2000

Group: Cell Biology 1

ABSTRACT

The Identification of Telomerase in Dog. (April 2000)

Anna Lyuksyutova
Department of Biology
Texas A&M University

Fellows Advisor: Dr. Dorothy Shippen
Department of Biochemistry and Biophysics

In recent years the enzyme telomerase has been found to be very active in human cancer cells but not in normal cells in the body. This discovery is predictable as telomerase is responsible for maintenance of the telomeres in a cell. It prevents the telomeres from diminishing during replication and ensures immortality to the cancerous cell. As the result, telomerase can be used as an identification test for malignant tissues. In humans, even though important, there are many other ways to determine if the tumour is malignant or benign. In animals, however, the alternative diagnostic methods are very expensive. Pet owners often decide against medical treatment and put the animal to sleep, even though in some cases the tumour itself is benign and not life threatening. The main goal of this project is to identify whether or not the telomerase plays the same role in dogs as it does in humans and whether an increase in telomerase activity is observed in dog cancers. The goal of this project is to propose an inexpensive and simple test for the presence of dog telomerase in tissues. This test will allow veterinarians to differentiate between benign and malignant tumours, making the diagnosis of the disease in dogs easier and less expensive.

ACKNOWLEDGEMENTS

I would like to thank Dr. Dorothy Shippen and everybody in the Shippen laboratory for their advice and support of my research project.

TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
INTRODUCTION.....	1
MATERIALS AND METHODS.....	3
RESULTS.....	6
DISCUSSION.....	9
CONCLUSION.....	11
BIBLIOGRAPHY.....	12
FIGURES.....	13
VITA.....	17

LIST OF FIGURES

FIGURE		Page
1a	Known sequence of cTR, location of cTR specific primers.....	13
1b	alligment of canine cTR with bovine and human cTR.....	13
2	SuperCos I cosmid restriction map.....	14
3	pUC 19 plasmid restriction map and multiple cloning site.....	14
4	Southern blot cTR probe preparation.....	15
5	Restriction digest of pUC 19 Pst I clones with Pst I	15
6	Amplification by PRC of cTR fragment from pUC 19 Pst I clones	15
7	Amplification by PCR of cTR from dog genomic DNA and SuperCosI.....	16
8	Amplification by PCR of cTR using nested primer combination.....	16

INTRODUCTION

Telomerase is the enzyme responsible for replication of short repeats found on the ends of all eukaryotic chromosomes - the telomeres. One of the first observers of telomeres was Barbara McClintock, who discovered that these structures were necessary to prevent chromosomes from breaking and sticking to each other in maize [1]. Still, telomeres are nothing more than long stretches of repetitive DNA, which do not carry any genetic information. Nevertheless the tips of the chromosomes play a very significant role in cell well-being [2].

In the 1970's James Watson pointed out a problem associated with DNA replication [1]. When the polymerase reached the end of the DNA strand, a short stretch of the lagging daughter strand would be lost, reducing one of the replicated chromosomes in size. If this happened with each replication, soon the chromosomes in a cell's progeny would disappear completely. This of course was not the case, as life must have found a way to restore the shrinking telomeres. In fact it was observed that in dividing cells chromosome telomeres were dynamic structures and could diminish or grow in size. Finally, an enzyme that was responsible for the restoration of the telomeres was identified – the telomerase [3]. First found in a ciliate, the telomerase was also discovered in many other species, in a highly conserved form. Recently, high telomerase activity had been identified in human tumors, which opened a new venue for the possible practical usage of this enzyme in human medicine [4].

A telomerase test for malignancies in human patients will be just one more way to diagnose cancer. Other methods of identification are available to medicine right now and are being used with much success. In animals, however, the alternative diagnostic methods are very expensive. For this reason pet owners often decide against medical treatment and put the animal to sleep, even though in some cases the tumour itself is benign and not life threatening. That is why new, inexpensive

diagnostic methods are necessary for veterinary patients [5]. Testing for telomerase could be a good alternative to computed tomography, which is usually unavailable or prohibitively expensive for diagnosis of veterinary patients.

Telomerase is also an important target for various chemotherapeutic drugs. To be able to use telomerase effectively for diagnosis or treatment it is essential to identify and study the subunits of this protein.

Telomerase consists of two subunits - the protein subunit which performs the polymerase activity, and the RNA subunit which serves as a template. The human telomerase RNA subunits and the telomerase RNA subunits of several other organisms have been identified and sequenced [6]. My main objective was to use the known stretch of the telomerase RNA subunit to obtain the complete sequence of the canine telomerase RNA (cTR) and to analyse telomerase activity and expression of cTR in canine tissue samples (see Fig. 1b). For this experiment a Northern blot of the appropriate tissue will be made, with the identified canine telomerase RNA subunit as the probe. The sequence of cTR can also be used for phylogenetic analysis to determine how far the gene is conserved among mammals.

The main goals of my research project were the isolation and sequencing of canine template RNA subunit (cTR) gene and comparison of the expression of the cTR and telomerase activity in different tissues. Another objective was to compare the expression of cTR and telomerase activity between malignant and benign samples of dog tissues.

MATERIALS AND METHODS

Southern Blot:

20ng of pUC19 cTR DNA were digested with various restriction enzymes and run out on a 0.6% agarose gel. The gel was washed in denaturing buffer for 20 min and for 30 min in neutralizing buffer. The gel was soaked in 2x SSC and left to blot overnight onto nylon membrane. Next the DNA on the membrane was x-linked twice to ensure it would not be washed away during hybridization. Radioactive probe for the hybridization reaction was prepared by PCR reaction (see Fig.4) using α -P³²-dGTP. 20 ul of the probe were boiled 3 min, then iced quickly and added to 50 ul of prehybe buffer (12.5ml 1M Na₂HPO₄, 0.1ml 0.5 M EDTA, 0.5g BSA, 17.5 ml 20% SDS, 19.9 ml H₂O). Southern blot membrane was prehybridized for ~2.5 hrs at 65°C in 100ml prehybe buffer. The prehybe buffer was discarded and the probe-containing buffer was added to the membrane, which was then hybridized overnight at 65°C. After hybridization was complete the probe was washed in 2xSSC at 65°C for 20 min, in 2xSSC + 1%SDS at 65°C for 20 min and 1xSSC + 1%SDS at 65°C for 20 min to overnight. The membrane was wrapped in plastic wrap and exposed to photographic film using intensifying screens for overnight.

Fast Link DNA Ligation kit (Epicentre, # LK63050)

The digested and gel-purified plasmid DNA suspected to contain cTR was mixed with opened pUC19 vector and other necessary components for a ligation reaction according to the Fast Link protocol. The reaction was incubated for 15 minutes at room temperature. After the incubation the ligase heat deactivated at 70°C for 15 min and then transformed into competent E.coli cells.

Cloning of plasmid vectors (Promega Protocols and Applications Guide 1996, p 46)

10ng of DNA were added to 200ul of competent cells and gently mixed. The reaction was incubated on ice for 30 min, then heat shocked at 42°C for 60 sec and placed on ice for 2 min. The

transformed bacteria were transferred to 3 ml of warm LB media (37°C) and incubated for 45 min at 37°C with shaking at ~150rpm. 50 -100 of the transformation mix plated on LB agar selection plates, which contained 40ug/ml X-Gal, 0.1mM IPTG and 100ug/ml ampicillin. The white colonies were picked and grown overnight in 3ml LB media at 37°C, then the DNA was isolated using the miniprep technique with Qiagen QIAprep Spin Miniprep Kit (Qiagen, #27104). The isolated clone's DNA was screened by PCR and restriction digest with EcoRI for the presence of the cTR gene (see Fig.5 and Fig.6).

BigDye® Sequencing (ABI, PE-ABI#4303150):

30 pmols of primer, 400ng template DNA, 8ul of BigDye® mix and H₂O were combined into a final volume of 20 ul. A sequencing PCR reaction was run (95°C for 10 sec, 50°C for 5 sec, 60°C for 4 min, 30 cycles) and the PCR product was purified using Biorad Micro Bio-Spin® Chromatography P-30 Column. The cleaned samples were then submitted to Gene Technologies Lab where they were run out on a sequencing gel.

Topo TA Cloning® of PCR Fragments (Invitrogen, #K4500-01)

dTR containing PCR fragments were obtained from dog genomic DNA (Clontech) using cTR primers R1 and F1 (see Fig.1a and Fig.7). These fragments were ethanol precipitated in order to clean the DNA from the rest of the PCR mix. Then, following the TOPO TA Cloning® protocol, 4ul of purified PCR product were mixed with 1ul Salt solution and 1 ul TOPO® vector. The reaction was incubated for 5 min at room temperature and 2 ul of the TOPO TA Cloning® reaction was added to One Shot® E. coli cells. The mix was incubated for 30 min on ice to ensure maximum transformation, then the cells were heat shocked for 30 sec at 42°C water bath, without shaking. The transformed cells were transferred into 250ul warm LB broth and incubated for 1 hr at 37°C with shaking at ~150rpm. All 250 ul were spread onto LB agar selection plates, which contained 40ug/ml X-Gal, 0.1mM IPTG,

100ug/ml ampicillin (Amp) and incubated overnight at 37°C. The white colonies were picked and screened by PCR and restriction digestion with EcoRI for the presence of the insert. (see [map of TOPO vector]).

Protein isolation from dog effusion samples:

An aliquot of 1 to 2 ml from each effusion sample was centrifuged (5000xg for 5 min) to obtain a cell pellet. The supernatant was discarded and the pellet combined with ice-cold lysis buffer in a 1/4 ratio. The samples were incubated on ice for 30 min and then centrifuged (12,000xg for 20 min at 4°C). Supernatants were aliquoted and stored at -80°C, while some of the protein extract was used for the Bradford assay in order to determine protein concentration in the sample.

Telomeric Repeat Amplification Protocol (TRAP)

TS primer was labeled with γ -³²P-ATP using T4 Polynucleotide Kinase (Promega). The labeled primer was then ethanol precipitated to clean the DNA from unincorporated radioactivity. The assay was setup following the Intergen Company protocol for TRAPeze assay (#S7700). 10 ng of protein extract were used per reaction. Each reaction was doubled up by a heat deactivated sample and positive and negative controls were included with each gel. The tubes were first incubated for 30 min at 30°C, then a 2-step PCR at 94°C /30 sec, 59°C /30 sec for 30 cycles was performed. The samples were separated on a 6% denaturing polyacrylamide gel electrophoresis (600V, 45min). The gel was dried and exposed overnight to photographic film.

RESULTS

Isolation of cTR

The bulk of the research efforts were directed on isolating and sequencing of the canine template RNA subunit (cTR) gene from a previously isolated canine genomic library clone. The cTR gene insert was in SuperCos vector (Stratagene) (see Fig.2) and has also been subclone into a smaller pUC19 plasmid (New England Biolabs) (see Fig.3). The insert was approximately 5.5kb in size. First objective was to isolate a smaller piece of the insert that contained cTR. The piece had to be small enough to be easily sequenced, however large enough to contain both the cTR gene and its promoter region, which sequence was also of interest. A variety of four and six base cutting restriction enzymes were used to digest the cTR containing pUC19 plasmid. Using the Southern blot technique 1 to 2 kb large pieces that would contain cTR for further work were identified. The probe for this experiment was prepared from PCR isolated cTR DNA (see Fig. 4) using TR specific primers R1 and F1 (see Fig.1a).

Subcloning of pUC19 fragments

The Southern blot experiments showed abundant hybridization. Two possible candidates were isolated: Hae II digested band of 2000 bp and Pst I digested band of 4000bp. Since pUC19 did not contain a convenient cloning site for Hae II, the larger size Pst I digested band was subcloned first. This 4kb insert was obtained by digesting pUC19 cTR DNA, separating the bands by gel electrophoresis and gel purifying the 4kb insert using the Concert™ Rapid Gel Extraction System (Life Technologies, 11456-019). The purified insert DNA was then ligated with an opened pUC19 vector using Fast Link™ DNA Ligation kit (Epicentre, # LK63050) and transformed into competent E.coli following the Promega protocol for transformation.

Screening of subcloned pUC19 fragments by PCR and their sequencing

The isolated clones were found to contain the insert of the appropriate size (see Fig. 5), however screening by PCR using several sets of cTR specific primers showed that none of the clones had the cTR sequence (see Fig. 6). Several other bands identified through other Southern blot experiments were also subcloned, however none of them gave positive results using PCR method. Nevertheless a sequencing experiment was attempted. For this the BigDye protocol from ABI was used.

cTR pUC 19 and SuperCos library clone do not contain cTR

The sequence obtained from the Gene Technologies Lab automated sequencing facility was scrambled and showed a very low signal. The conditions of the sequencing reaction were adjusted several times and a new set of primers was ordered from Genesys (R3, F3, F4). While the control sequencing reaction was now working nicely the subcloned cTR pUC19 DNA didn't gave good sequence. After numerous trials with new subclones and varying PCR conditions I decided to use dog genomic DNA (Clontech, #6950-1) as a control with my specific cTR primers. I also checked the cosmid clone, from which the 5.5kb insert suspected to contain cTR was isolated. Using PCR protocol I checked the cosmid DNA with cTR specific primers R1 and F1 together with a positive PCR control (an empty pUC19 with standard M13 primers) and genomic DNA. While the positive control and genomic DNA showed bands of appropriate length there was no band in the cosmid PCR reaction (see Fig. 7).

Preparation of cTR probe from dog genomic DNA

The next goal was to isolate a new clone of cTR from a dog genomic library. However, before library screening experiment was performed, a probe had to be constructed that could be used to screen the cosmid library for the cTR gene. For this purpose dog genomic DNA was amplified using cTR specific primers R1 and F1 (see Fig. 7). The obtained fragment was checked once again

through PCR using a nested set of cTR specific primers R1 and F4 (see Fig. 1a and Fig. 8). Instead of a 250bp fragment that is amplified by the R1/F1 pair, the primer pair of R1/F4 produces a 200bp long fragment which is located within the sequence amplified by R1/F1 (see Fig. 1a). This experiment also turned out to be positive. After receiving satisfactory results from PCR the amplified fragments were ethanol purified and subcloned into TOPO vector using the TOPO TA Cloning kit.

Optimization of the Telomeric Repeat Amplification Protocol (TRAP)

Concurrently with cTR probe preparation the second objective of the research project was pursued. Together with Raquel Brown, a veterinary doctor from the lab of Dr. Kenita Rogers I was analyzing telomerase activity in canine effusion samples from cancerous and benign tumors as well as cancer unrelated conditions, such as heart disease. Currently the conditions of the TRAP assay for the effusion samples are being adjusted. However good results from positive controls have already been obtained.

DISCUSSION

Isolation of cTR

The subcloning strategy was chosen since it was necessary to obtain the cTR sequence as soon as possible. A smaller insert would allow a more rapid sequencing of the cTR gene. The hybridization probe was prepared from PCR amplified cTR DNA that was obtained from genomic DNA. Several Southern blots were prepared with about 10 different restriction digests on each. However, most of the restriction enzymes didn't digest the plasmid very well. Also, especially for the second Southern blot the problem of unspecific probe binding was encountered, as practically everything on the blot was binding the probe, including the DNA markers. At first a mistake in the probe preparation was considered to be the cause of non specific binding. However, when a new probe was prepared and the experiment repeated the probe was still binding very unspecifically. Thus the problem was not with the mistake in probe preparation but the DNA that was used to prepare the probe. I am currently working on preparing a more reliable probe for cTR.

Subcloning of pUC19 fragments

Southern blot experiments identified several possible candidates for subcloning. The two most likely candidates were HaeII 2kb fragment and PstI 4 kb fragment. However, HaeII was not part of the multiple cloning site of the pUC19 vector. Also, HaeII was a sticky end cutter with a rare sequence recognition. Thus, even though PstI fragment was substantially larger than the preferred length of 1-2 kb this fragment was subcloned. PstI is part of the multiple cloning site and can be easily inserted into pUC19.

cTR pUC 19 and SuperCos library clone do not contain cTR

While both Southern blots showed abundant hybridization, after the transformed DNA was isolated and screened for insert cTR gene was not found by PCR, though the isolated plasmids contained inserts of the right size. When sequencing of the insert was attempted the sequence turned out scrambled, with a very low signal, which is a sign of either poor DNA template or poor primer binding. These behaviours of the clone lead me to think that the clone was empty, e.g. did not contain the desired cTR sequence, but instead some other part of the dog genomic library. The cosmid and dog genomic DNA were compared by PCR (see Fig.7), which showed no signal for the cosmid clone, while there was a visible band for the genomic DNA PCR reaction. After this follow up analysis it seemed that the dog library clones identified were empty and the probe hybridization must have been non-specific. Alternatively a wrong clone could have been picked up from the dog genomic library screen.

Preparation of cTR probe from dog genomic DNA

Currently I am repeating the experiment, trying to isolate the cTR from a dog library. The knowledge of the cTR sequence is necessary to continue my project. I plan to examine dog tissue using Northern blot technique to compare the dog telomerase template RNA levels in various dog tissues, as well as in malignant samples. The sequence of the dog template RNA gene can also be used for comparison with other known cTR sequences in order to analyse how far this gene is conserved among mammals.

CONCLUSION

This research project investigates two very important areas. It will allow to better understand the role of telomerase in cancerous tumours, as well as research the characteristics of telomerase in a new animal model, the dog. It has been suggested that dog is a better telomerase study model than the more commonly used mouse, since dog telomerase behaves much more like human telomerase, while in mice its expression patterns are significantly different [7]. Upon completion my research project will contribute to the basis for developing of a new simple and accurate diagnostic test for veterinary patients with cancers. This project will also provide new knowledge concerning the relationship between telomerase, tumour genesis and cellular aging in mammals.

BIBLIOGRAPHY

1. Greider, C.W. and E. H. Blackburn, (1996) Scientific American (February): 80 – 85.
2. Blackburn E.H. (1991) Trends in Biochemical Science 16: 378-381
3. Greider, C.W. and E. H. Blackburn, (1985) Cell 43: 405-413
4. Kim, N.W. et al. (1994) Science 266: 2011-2019
5. Shippen, D. and K. Rogers, (1999) Interdisciplinary Research Initiatives Program for TAMU

Research Enhancement Programs.

6. Greider C.W. et al. (2000) Cell 100: 503-514
7. Tsujimoto H. et al. (1999) Journal of Veterinary Medical Science 61(10): 1125-1129

BIBLIOGRAPHY

1. Greider, C.W. and E. H. Blackburn, *Scientific American* (**February**), 80 – 85, (1996)
2. Blackburn E.H. *Trends in Biochemical Science* **16**, 378-381, (1991)
3. Greider, C.W. and E. H. Blackburn, *Cell* **43**, 405-413, (1985)
4. Kim, N.W. et al. , *Science* **266**, 2011-2019, (1994)
5. Shippen, D. and K. Rogers, *Interdisciplinary Research Initiatives Program for TAMU _Research Enhancement Programs*, (1999)
6. Greider C.W. et al. *Cell* **100**, 503-514, (2000)
7. Tsujimoto H. et al. *Journal of Veterinary Medical Science* **61(10)**, 1125-1129, (1999)

Figure1 (a&b):

5' end- cgcgctgtttttctcgctgactttcagcgggcggaagcctcgccct

gcgcgcttccacggttcagctctagagcaaaacaaaaatgtcagccgct
 -----F1----->
 -----F4----->
 ggctcgctcacctctcccgggaacctgcggtgggtccgcccgcagcc

ccagtgccccgcctgaggccgcggtcgcccggtgcttctccggaggt

gtccattgccgcctgaagagtggtggctctgtcaaccgcgggtcgctc

ggtggggccgagggcatggctgtaaccgcagggaaaggaacggagtggg
 <-----R1-----
 -----R3----->

gtccccgcgcggtgcgcttccctgagctgtgggaattgcacc - 3' end

Known sequence of canine telomerase RNA subunit (cTR). Sequence specific primers are shown. The distance between R1 and F1 is ca. 250 bp. The distance between F4 and R1 is ca. 200 bp. R3 was used for sequencing out to the 3' end.(2)

human	GGGTTCGGGA	GGGTGGCCCT	GGGAGGGGTG	GTGGCCATT	TTTGCTAAC	CCTAACTGAG	AAGGCG	GTA
bovine	GA	GGGTGGGCC	CGGGTTGGTG	GCAGCCATT	CATATCTAAC	CCTAATTGAG	ACAGCG	GTA
canine								
human	GGGGCCGTGC	TTTTGCTCC	CGCGCGCTGT	TTTTCTGCT	GACTTTCAGC	GGG	CGGAAA	AGCCTCGGCC
bovine	GGGGCTGTGC	TTTTGCTTAC	CGCGCGCTGT	TTTTCTGCT	GACTTTCAGC	GGG	CGGAAA	AGCCTCGGCC
canine			<u>CGCGCTGT</u>	<u>TTTTCTGCT</u>	<u>GACTTTCAGC</u>	GGG	CGGAAA	AGCCTCGGCC
human	TGCGCCCTTC	CACCGTTTAT	TCTAGAGCAA	ACAAAAAATG	TCAGTGTCTG	GCCCGTTCGC	CTCCCGGG	
bovine	TACCGCCATC	CACCATCCAG	TCTGCAACAA	ACAAAAAATG	TCAGCCGCTG	GCTCGCTCAC	CTCTCCCGGG	
canine	TGCGCCCTTC	CACCGTTTAC	TCTAGAGCAA	ACAAAAAATG	TCAGCCGCTG	GCTCGCTCAC	CTCTCCCGGG	
human	GACCTCGCGC	GGTGCCTCTG	CCCAGCCCCC	GAACCCCGCC	TGGAGCCGCG	GTGCGCCCGG	GGCTTCTCCG	
bovine	AACCTCGCGT	GGTGCCTCTG	CCCAGCCCCA	GTGCCCGGCC	TGAGCCCGCG	GTGCGCCCGG	TGCTTCTCCG	
canine	AACCTCGCGT	GGTGCCTCTG	CCCAGCCCCA	GTGCCCGGCC	TGAGCCCGCG	GTGCGCCCGG	TGCTTCTCCG	
human	GAGGCACCCA	CTGCCACCGC	GAAGAGTTGG	GCTCTGTCTG	CCGCGGGTCT	CTCGGGGGGG	AGGCGAGGTT	
bovine	GAGGTGTCCA	TTGCCCGCGT	GAAGAATTGG	GCTCTGTCTG	CCGCGGGTCT	CTCGGGGGGG	AGGCGAGGTT	
canine	GAGGTGTCCA	TTGCCCGCGT	GAAGAGTTGG	GCTCTGTCTG	CCGCGGGTCT	CTCGGGGGGG	AGGCGAGGTT	
human	TCACCGTTTC	AAGCCGCGAG	AAGAGGAACG	GAGCGAGTCC	CGCCGCGCGC	CGATTCCCTG	AGCTGTGGGA	
bovine	ATGGCTGTTA	CCGCAAGGAA	AGGAACGGAG	TGGGGTCCCC	CGCGCGGGTG	CGCTTCCCTG	AGCTGTGGGA	
canine	ATGGCTGTAA	CCGCAAGGAA	AGGAACGGAG	TGGGGTCCCC	CGCGCGGGTG	CGCTTCCCTG	AGCTGTGGGA	
human	CGTGCACCCA	GCACTCGGCT	CACACATGC					
bovine	CTTGCAACCG	GCACTCGGCT	CAGACATCC					
canine	<u>ATTGCACCC</u>							

Figure 1. Alignment of telomerase RNA (TER) subunits from human, bovine and canine. PCR primers used to amplify the canine sequence are indicated by underlining. The 5' and 3' terminal sequences for the canine RNA will be determined when obtain a full-length clone of the gene is available.

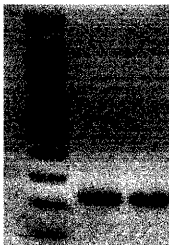


Figure 4:

In earlier experiments I was able to isolate fragments of expected size (250bp) for probe preparation using PCR. Lane 1 contains a 100bp ladder, while lane 2 and 3 contain PCR reactions using isolated dog TR DNA and specific dTR primers R1 and F1.

Figure 5:

The subcloned Pst I fragment was analyzed using restriction enzyme digest. The pUC19 + insert plasmid was digested with Pst I. The upper band is the insert, which is 4 kb in size. The lower band is pUC19 which is 2.7kb in size. This gel electrophoresis shows that the ligation and transformation reactions were successful.

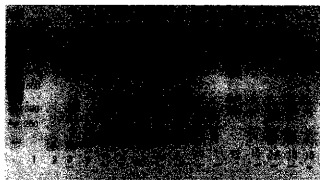


Figure 6:

The pUC 19 + PstI insert plasmid was checked by PCR using cTR specific set of primers R1/F1. Multiple bands that are observed on this gel show that there was no specific primer binding during the reaction. A strong band in the 100 bp region points to primer dimers. This gel shows that, while the transformation experiment was successful the insert that was transformed into pUC 19 did not contain cTR.

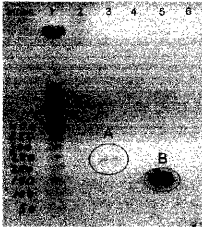
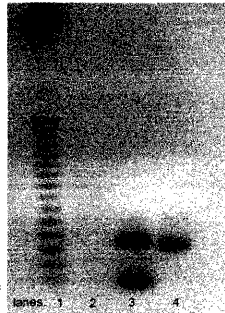


Figure 7:

PCR reaction was performed using genomic DNA (lane3) and cosmid dTR clone DNA(lane6) using dTR Forwar1 and Reverse1 primers , as well as on an empty puc19(lane5) vector using standard M13 forward and reverse primers as a PCR control. The control lane (lane 5) shows a bright band(B), thus PCR worked. There is a fainter band(A) for genomic DNA, which is 250 bp in size, as expected for this set of primers. However the cosmid PCR reaction doesn't show any band. This means that the cosmid clone pulled from the library doesn't contain dTR. Lane 1 contains a 50bp DNA ladder (Biorad).

Figure 8:

PCR reaction was performed on the isolated dTR PR product, which was obtained using dog genomic DNA and specific dTR primers. This reaction was done in order to ensure that the fragment isolated was indeed dTR. In lane 3 the reaction was repeated using dTR F1 and R1 primers, which give a 250 bp product, while in lane 4 a new set of primers, F4 and R1 were used. These primers give a 200 bp long fragment. The gel electrophoresis showed CR fragments of the indicated length, proving that the fragments isolated are dTR. The lower band in the lane 3 is due to primer dimers.



VITA**ANNA LYUKSYUTOVA**

613 Peyton

College Station, TX 77840

EDUCATION

Bachelor of Science in Molecular Biology

Texas A&M University, College Station

Degree expected May, 2000

AWARDS

- * Academic Achievement Award (merit based scholarship available for international students, \$1000) [academic years '98 -'99 and '99 - '00]
- * Dean's Honor Roll (GPR above 3.75) [Fall'97, Spring'98], Distinguished Student [Summer'98],
- * University Honors Research Fellow ['99 - '00]
- * will be graduating with University and Foundation Honors
- * member of Golden Key Honor Society, Phi Eta Sigma Honor Society and Sigma Xi Honor Society
- * Memorial Student Center Distinguished Student of the Month [March '99]